

## Research Article

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# Induced resistance to *Helicoverpa armigera* through exogenous application of jasmonic acid and salicylic acid in groundnut, *Arachis hypogaea*

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## Abstract

**BACKGROUND:** Induced resistance to *Helicoverpa armigera* through exogenous application of jasmonic acid (JA) and salicylic acid (SA) was studied in groundnut genotypes (ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697) with different levels of resistance to insects and the susceptible check JL 24 under greenhouse conditions. Activities of oxidative enzymes and the amounts of secondary metabolites and proteins were quantified at 6 days after JA and SA application/insect infestation. Data were also recorded on plant damage and *H. armigera* larval weights and survival.

**RESULTS:** Higher levels of enzymatic activities and amounts of secondary metabolites were observed in the insect-resistant genotypes pretreated with JA and then infested with *H. armigera* than in JL 24. The insect-resistant genotypes suffered lower insect damage and resulted in poor survival and lower weights of *H. armigera* larvae than JL 24. In some cases, JA and SA showed similar effects.

**CONCLUSION:** JA and SA induced the activity of antioxidative enzymes in groundnut plants against *H. armigera*, and reduced its growth and development. However, induced response to application of JA was greater than to SA, and resulted in reduced plant damage, and larval weights and survival, suggesting that induced resistance can be used as a component of pest management in groundnut.

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**Keywords:** groundnut; *Helicoverpa armigera*; induced resistance; secondary metabolites; antioxidant enzymes; jasmonic acid; salicylic acid

## 1 INTRODUCTION

Plants have developed an elegant defence system against insect herbivory. The defence systems employed by plants against insects can be constitutive or induced. Constitutive resistance is present in plants all the time, whereas induced resistance occurs in response to various stimuli such as insect herbivory, pathogen infection and/or elicitor application.<sup>1–3</sup> Induced resistance is very important as it makes plants phenotypically plastic, thereby making it freakish for the insect pests to feed on it.<sup>4,5</sup> Induced resistance can be direct or indirect. Direct induced resistance directly affects the insect pest through antixenosis and/or antibiosis mechanisms,<sup>6,7</sup> whereas indirect induced resistance is mediated through volatiles emitted by the plants in response to insect damage, which attract the natural enemies (parasitoids and predators) of the insect pests.<sup>4,8,9</sup>

Although many plant hormones act as elicitors of induced resistance, the most important and widely used phytohormones are jasmonic acid (JA) and salicylic acid (SA).<sup>3,10</sup> The use of these phytohormones in inducing plant resistance against insect pests has raised the possibility of their implications for insect pest management. Exogenous application of JA results in the induction

of plant responses that are almost similar to herbivore feeding. The JA-mediated octadecanoid pathway leads to the production of many defensive components, such as plant defensive proteins, oxidative enzymes, glandular trichomes, flavonoids, terpenoids, alkaloids, volatile compounds, etc.<sup>1,4,9</sup> SA, a benzoic acid derivative, is an endogenous plant growth regulator that generates in plants a wide range of metabolic and physiological responses involved in plant growth and development,<sup>11</sup> and defence against various stresses, including insect herbivory.<sup>3,10,12</sup>

Groundnut (*Arachis hypogaea* L.) is an annual herbaceous plant belonging to the family Fabaceae. It is cultivated mostly in semi-arid tropical and subtropical regions. It is damaged by several

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insect pests, of which the legume pod borer, *Helicoverpa armigera* (Hübner), is an important defoliator during the vegetative stage. *H. armigera* is widely distributed in Asia, Africa, southern Europe and Australia.<sup>13</sup> In semi-arid tropics, *H. armigera* causes an estimated loss of over \$US 2 billion annually, in spite of the \$US 500 million worth of pesticides applied for controlling this pest.<sup>13</sup> It has developed high levels of resistance to several commonly used insecticides.<sup>14</sup> Therefore, there is a need for alternative methods of pest control to reduce overdependence on insecticides and to conserve biodiversity. It is in this context that host plant resistance, which is economic and environmental friendly, assumes a central role in integrated pest management.<sup>13</sup>

Host plant resistance plays an important role in groundnut defence against a variety of insect pests. Many biochemical parameters have been associated with resistance in groundnut against insect pests. Higher levels of antioxidative enzymes, phenols and tannins contribute to groundnut resistance against *Spodoptera litura* (Fab.) and *H. armigera*.<sup>15–18</sup> Stevenson *et al.*<sup>15</sup> observed that quercetin, caffeoylquinic acids and diglycosides contribute to resistance in groundnut against *S. litura*. Procyanidin in groundnut plants provides resistance against *Aphis craccivora* (Koch).<sup>16,19</sup> Nitrogen, soluble sugars and polyphenols are involved in groundnut resistance against leaf miner *Aproraema modicella* Dev.<sup>20</sup> Understanding the mechanisms of induced resistance can help in building up the natural defences in plants by the application of elicitors and/or mild damage by the herbivores. Although it has been well documented that phytohormones induce plant resistance in plants through the expression of a number of proteins and non-protein-based compounds, such studies are limited in groundnut. To test this hypothesis, JA and SA were exogenously applied to groundnut plants with differential levels of resistance to *H. armigera* to study the induced resistance. The plants were pre- and/or simultaneously treated with JA and SA and infested with *H. armigera*. Various plant defensive enzymes and plant secondary metabolites were investigated.

## 2 MATERIALS AND METHODS

### 2.1 Chemicals

The chemicals used in this study were of analytical grade. Ethylene diamine tetraacetic acid (EDTA), bovine serum albumin (BSA), guaiacol, polyvinylpyrrolidone (PVP), jasmonic acid, salicylic acid, tannic acid, vanillin, linoleic acid, dithiothreitol (DTT), disodium hydrogen phosphate, sodium dihydrogen phosphate, nitro-blue tetrazolium salt (NBT), methionine, L-phenylalanine, sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and vanillin were obtained from Sigma Aldrich, St Louis, Missouri. Catechol was obtained from Glaxo Laboratories, Mumbai, India. Tris-HCl, glycine and trichloroacetic acid (TCA) were obtained from Sisco Research Lab., Mumbai, India. 2-Mercaptoethanol, gallic acid and Folin-Ciocalteu reagent were obtained from Merck, Mumbai, India. Thiobarbituric acid (TBA) and linoleic acid were obtained from HiMedia Pvt. Ltd, Mumbai, India. Ammonium sulphate was obtained from Qualigens Fine Chemicals, Mumbai, India. The spectrophotometer used for the estimation of biochemical parameters was a Hitachi UV-2900 (Hitachi, Tokyo, Japan).

### 2.2 Groundnut plants

Five groundnut genotypes were grown under greenhouse conditions at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, to study the

induction of resistance by exogenous application of JA and SA against *H. armigera*. The genotypes were ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 (with moderate levels of resistance to insects) and JL 24 (susceptible check).<sup>21</sup> The plants were raised in plastic pots (30 cm diameter and 39 cm deep) containing a mixture of soil, sand and farmyard manure (2:1:1). Five seeds were planted in each pot, and two seedlings were retained in each pot at 5 days after seedling emergence. Desert coolers were used to maintain the temperature at 28 ± 5 °C and RH 65 ± 5% in the greenhouse. After 20 days of emergence, plants were infested with ten newly emerged *H. armigera* larvae with a camel hair brush. The experiment was repeated 3 times, and the data shown are the pooled data.

### 2.3 Insect infestation

The *H. armigera* neonates were obtained from the stock culture maintained on chickpea-based semi-synthetic diet<sup>22</sup> under laboratory conditions (26 ± 1 °C, 11 ± 0.5 h photoperiod, 75 ± 5% relative humidity) from the insect rearing laboratory at ICRISAT, Patancheru, Andhra Pradesh, India.

### 2.4 Induction of resistance by exogenous application of JA and SA in groundnut against *H. armigera*

There were six treatments for each genotype and five replications for each treatment, with two plants in each replication. In group I the plants were pretreated with JA (1 mM) for 24 h and then infested with *H. armigera* (PJA + HIN); in group II the plants were pretreated with SA for 24 h and then infested with *H. armigera* (PSA + HIN); in group III the plants were sprayed with JA (1 mM) and simultaneously infested with *H. armigera* (JA + HIN); in group IV the plants were sprayed with SA (1 mM) and simultaneously infested with *H. armigera* (SA + HIN); in group V the plants were infested with *H. armigera* (HIN); in group VI the plants were maintained as untreated control (sprayed with ethanol only).

At 6 days after treatment, plants were assessed for insect damage by visually rating them to a scale of 1–9, with 1 showing no or slight damage (<10%) and 9 shows >80% damage.<sup>21</sup> Larvae recovered from the plants were counted and weighed to record data on insect survival and larval weights.

The fully expanded quadrifoliate leaves were collected randomly from the groundnut plants at 6 days after treatment to study the activities of various defensive enzymes such as peroxidase (POD), polyphenol oxidase (PPO), lipoxygenase (LOX), phenylalanine ammonia lyase (PAL), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), trypsin proteinase inhibitor (PI) and total amounts of phenols, condensed tannins, flavonoids, carbohydrates, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA).

#### 2.4.1 Enzyme extraction

Fresh leaves (0.5 g) were ground in 3 mL of ice-cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5 mM of 2-mercaptoethanol, 1% polyvinylpyrrolidone (PVP), 1 mM of DTT and 0.5 mM of EDTA. The homogenate was centrifuged at 14 000 rpm for 20 min, and the supernatant was collected. The supernatant was subjected to protein precipitation using ammonium sulphate (NH<sub>4</sub>SO<sub>4</sub>) and dialysed using a dialysis bag (Sigma-Aldrich).

#### 2.4.2 Enzyme assays

Activities of enzymes such as peroxidase,<sup>23</sup> polyphenol oxidase,<sup>24</sup> lipoxygenase<sup>25</sup> and SOD<sup>26</sup> were estimated by adopting standard

procedures. The enzyme activity was expressed as units per gram fresh weight ( $\text{IU g}^{-1} \text{FW}$ ). One unit of enzyme was defined as the change in absorbance by 0.1 unit per minute under conditions of the assay. Phenylalanine ammonia lyase was estimated as described by Campos-Vergas and Saltveit,<sup>27</sup> with slight modifications. The enzyme activity was expressed as  $\mu\text{mol cinnamic acid min}^{-1} \text{mg}^{-1} \text{protein}$ . Catalase activity was determined by using the method of Zhang *et al.*<sup>28</sup> and the enzyme activity was expressed as  $\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$ .

APX activity was determined by the method of Asada and Takahashi.<sup>29</sup> Leaf tissue (0.2 g) was homogenised in a pestle and mortar with 3 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM of EDTA, 1% polyvinylpyrrolidone (PVP) and 1 mM of ascorbic acid. After filtering through a double-layered cheesecloth, the homogenate was centrifuged at 12 000 rpm for 20 min at 4°C. The supernatant was collected and subjected to precipitation and dialysis as mentioned above. The partially purified sample was used as the enzyme source. The reaction mixture (1 mL) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM of ascorbic acid, 0.1 mM of  $\text{H}_2\text{O}_2$  and 0.2 mL of partially purified enzyme extract. Decrease in absorbance at 290 nm owing to ascorbate oxidation was measured against the blank, and the enzyme activity was expressed as  $\text{IU g}^{-1} \text{FW}$ .

#### 2.4.3 Proteinase inhibitor (PI) activity

To measure PI activity, the leaf sample (0.2 g) was homogenised in 4 mL of 50 mM Tris-HCl buffer (pH 7.8) containing 5% PVP, 0.016 M of phenyl urea, 0.03 M of KCl, 0.05 M of EDTA and 0.4 mM of ascorbic acid. The homogenate was filtered through three layers of cheesecloth and centrifuged at 12 000 rpm for 15 min at 4°C. The supernatant was collected, and the protein was precipitated with ammonium sulphate, dialysed and used as the protein inhibitor source. All the steps were carried out on ice to ensure the lowest possible temperature. The PI activity was estimated by following the method of Kakade *et al.*<sup>30</sup> using *N*- $\alpha$ -benzoyl-DL-arginyl-*p*-nitroanilide (BAPNA) as a substrate, and trypsin as a standard. The PI activity was expressed as percentage inhibition of trypsin.

#### 2.4.4 Estimation of secondary metabolites and other defensive compounds

**Phenolic content.** Fresh leaves (0.5 g) were homogenised in 3 mL of 80% methanol and agitated for 15 min at 70°C. The solution was centrifuged at 10 000 rpm for 10 min, and the supernatant was collected. The supernatant was used for estimation of total phenols, condensed tannins and total flavonoids. The phenolic content was estimated by the method of Zieslin and Ben-Zaken.<sup>31</sup> The amounts of total phenols were determined from the standard curve prepared with gallic acid, and expressed as  $\mu\text{g gallic acid equivalents g}^{-1} \text{FW}$  ( $\mu\text{g GAE g}^{-1} \text{FW}$ ). The condensed tannin content was estimated using the vanillin-hydrochloride method as described by Robert.<sup>32</sup> Catechin was used as the standard. The total amount of condensed tannins was expressed as  $\mu\text{g catechin equivalents g}^{-1} \text{FW}$  ( $\mu\text{g CE g}^{-1} \text{FW}$ ). The total flavonoid content was determined by the modified aluminium chloride method as described by Woisky and Salatino.<sup>33</sup> The total amount of flavonoids was expressed as  $\mu\text{g CE g}^{-1} \text{FW}$ .

**Hydrogen peroxide.** The hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) content was estimated by the method of Noreen and Ashraf.<sup>34</sup> The  $\text{H}_2\text{O}_2$  concentration was determined by using an extinction coefficient of  $0.28 \mu\text{M cm}^{-1}$  and expressed as  $\mu\text{mol g}^{-1} \text{FW}$ .

**Malondialdehyde.** The level of lipid peroxidation was determined in terms of thiobarbituric-acid-reactive substances (TBARSs), i.e. MDA, as described by Carmak and Horst,<sup>35</sup> with minor modifications. The concentration of TBARSs was calculated using an absorption coefficient of  $155 \text{ mmol}^{-1} \text{cm}^{-1}$  and expressed as  $\mu\text{mol g}^{-1} \text{FW}$ .

**Protein content.** The total protein content was estimated by Lowey method<sup>36</sup> using bovine serum albumin as a standard.

### 2.5 Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SPSS (v.15.1; SPSS Inc., Chicago, IL). Tukey's multiple comparison test was used to separate the means when the treatment effects were statistically significant ( $P \leq 0.05$ ).

## 3 RESULTS

### 3.1 Induction of enzyme activity and secondary metabolites following exogenous application of JA and SA in groundnut

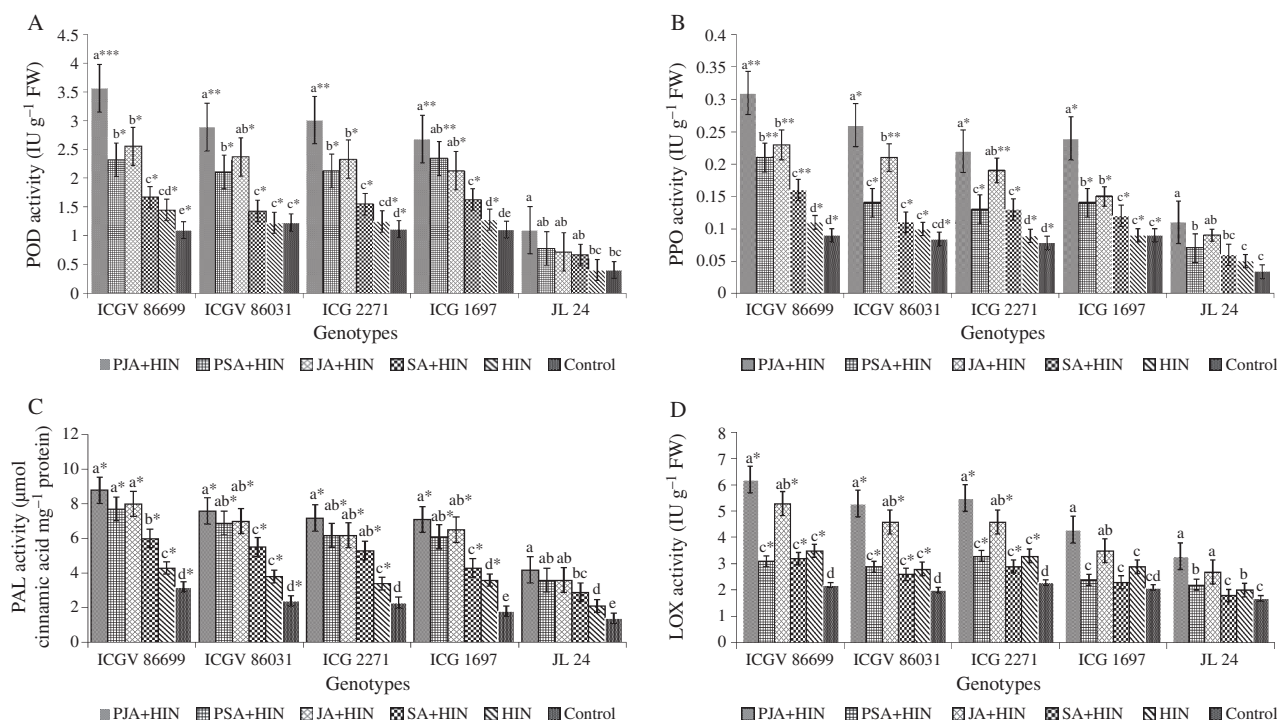
#### 3.1.1 POD activity

The PJA + HIN-treated plants showed significantly greater POD activity in ICGV 86699 and ICG 2271 ( $F_{5,17} = 23.4$  and  $48.1$ , respectively,  $P < 0.01$ ) than PSA + HIN-treated, JA + HIN-treated, HIN-treated and untreated control plants (Fig. 1A). In ICGV 86031, PJA + HIN-treated and JA + HIN-treated plants exhibited significantly greater POD activity ( $F_{5,17} = 27.4$ ,  $P < 0.05$ ) than PSA + HIN-treated, SA + HIN-treated, HIN-treated and untreated control plants; however, POD activity of PSA + HIN-treated plants was at par with that of JA + HIN-treated plants. In ICG 1697 and JL 24, plants treated with PJA + HIN, PSA + HIN and JA + HIN showed significantly greater POD activity ( $F_{5,17} = 29.3$  and  $18.1$ , respectively,  $P < 0.05$ ) than SA + HIN-treated, HIN-treated and untreated control plants. Across the genotypes, insect-resistant genotypes showed significantly greater POD activity in all the treatments ( $F_{4,14} = 36.8$ ,  $15.0$ ,  $19.6$ ,  $9.9$  and  $12.6$  for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and the control, respectively,  $P < 0.05$ ) than the susceptible check JL 24.

#### 3.1.2 PPO activity

Among the treatments, PJA + HIN-treated plants had significantly greater PPO activity in ICGV 86699 ( $F_{5,17} = 25.7$ ,  $P < 0.01$ ), ICGV 86031 ( $F_{5,17} = 23.4$ ,  $P < 0.01$ ) and ICG 1697 ( $F_{5,17} = 11.9$ ,  $P < 0.05$ ) than the plants treated with PSA + HIN, JA + HIN and SA + HIN, *H. armigera*-infested plants and the untreated control plants (Fig. 1B). In ICG 2271, plants infested with *H. armigera* and pre- and/or simultaneously treated with JA showed significantly greater PPO activity ( $F_{5,17} = 20.1$ ,  $P < 0.05$ ) than SA + HIN-treated, HIN-treated and untreated control plants. In JL 24, no significant difference was recorded in PPO activities of plants treated with PJA + HIN and JA + HIN ( $F_{5,17} = 18.7$ ,  $P < 0.05$ ); however, PPO activity of JA + HIN-treated plants was at par with that of PSA + HIN-treated and SA + HIN-treated plants. Among the tested genotypes, ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 had significantly higher PPO activity in PJA + HIN-treated plants ( $F_{4,14} = 16.7$ ,  $P < 0.05$ ) than in JL 24. The PSA + HIN-treated plants of insect-resistant genotypes showed significantly greater PPO activity than JL 24; however, the level of significance varied [ICGV 86699 ( $P < 0.001$ ) and ICGV 86699, ICG 2271 and ICG 1697 (all  $P < 0.05$ )]. Significantly greater PPO activity was observed in JA + HIN-treated plants of ICGV 86699, ICGV 86031 and ICG 2271





**Figure 1.** Enzyme activities of groundnut plants pre- and/or simultaneously treated with JA and SA and infested with *H. armigera*: (A) peroxidase (POD) activity ( $\text{IU g}^{-1} \text{FW}$ ); (B) polyphenol oxidase (PPO) activity ( $\text{IU g}^{-1} \text{FW}$ ); (C) phenylalanine ammonia lyase (PAL) activity ( $\mu\text{mol cinnamic acid min}^{-1} \text{mg}^{-1} \text{protein}$ ); (D) lipoxygenase (LOX) activity ( $\text{IU g}^{-1} \text{FW}$ ). Bars (mean  $\pm$  SD) of the same colour with similar letters within a genotype are not statistically different at  $P \leq 0.05$ . Asterisks on bars of the same colour show the significance across the genotypes within a treatment: \*\*\*, \*\*, \* = significant at  $P \leq 0.001$ ,  $0.01$  and  $0.05$ , respectively. PJA + HIN = pretreatment with JA 1 day prior to *H. armigera* infestation; PSA + HIN = pretreatment with SA 1 day prior to *H. armigera* infestation; JA + HIN = simultaneous application of JA and *H. armigera* infestation; SA + HIN = simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera*-infested plants.

( $F_{4,14} = 22.5$ ,  $P < 0.05$ ) compared with those of ICG 1697 and JL 24. Constitutive levels of PPO activity were significantly higher in insect-resistant genotypes ( $F_{4,14} = 8.9$ ,  $P < 0.05$ ) than in JL 24.

### 3.1.3 PAL activity

The PJA + HIN-treated, PSA + HIN-treated and JA + HIN-treated plants showed significantly greater PAL activity ( $F_{5,17} = 45.7$ ,  $22.9$  and  $16.9$  for ICGV 86699, ICGV 86031 and ICG 1697, respectively,  $P < 0.05$ ) than the SA + HIN-treated, HIN-treated and untreated control plants (Fig. 1C). In ICG 2271 and JL 24, the PAL activity of plants treated with PSA + HIN and SA + HIN did not differ significantly. Among the genotypes tested, ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 exhibited significantly greater PAL activity in PJA + HIN-treated, PSA + HIN-treated, JA + HIN-treated, SA + HIN-treated, HIN-treated and untreated control plants ( $F_{4,14} = 21.8$ ,  $11.9$ ,  $32.5$ ,  $17.9$ ,  $28.4$  and  $16.4$ , respectively,  $P < 0.01$ ) compared with JL 24.

### 3.1.4 LOX activity

The plants infested with *H. armigera* and pre- and/or simultaneously treated with JA showed significantly greater LOX activity in all the genotypes tested ( $F_{5,17} = 32.5$ ,  $21.3$ ,  $23.9$ ,  $21.9$  and  $13.2$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.05$ ) than the plants treated with PSA + HIN, SA + HIN and HIN and the untreated control plants (Fig. 1D). ICGV 86699, ICGV 86031 and ICG 2271 plants treated with PJA + HIN, PSA + HIN, JA + HIN and HIN showed significantly greater LOX activity ( $F_{4,14} = 32.1$ ,  $24.6$ ,  $18.4$  and  $14.3$ , respectively,  $P < 0.01$ ) than the respective

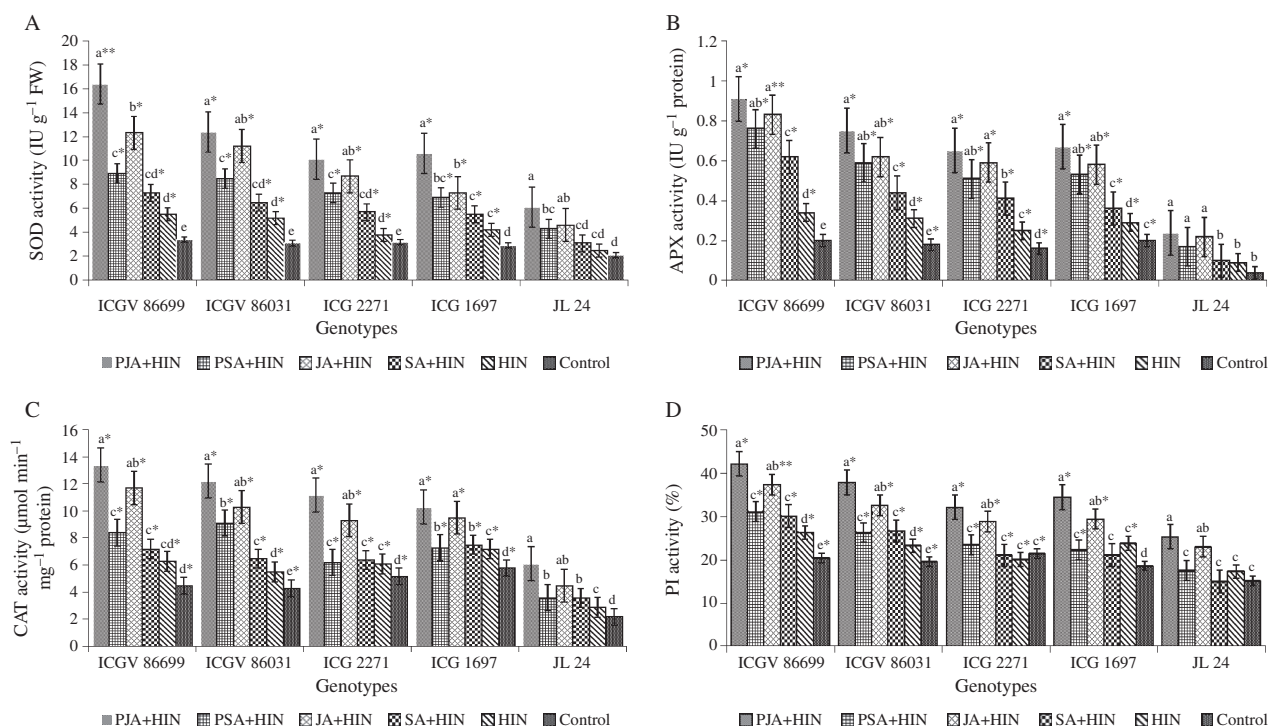
treatments of ICG 1697 and JL 24. No significant differences were observed in the LOX activity of untreated control plants.

### 3.1.5 SOD activity

The PJA + HIN-treated plants had significantly greater SOD activity in ICGV 86699 and ICG 1697 ( $F_{5,17} = 11.3$  and  $15.2$ , respectively,  $P < 0.05$ ) than PSA + HIN-treated, JA + HIN-treated, SA + HIN-treated, HIN-treated and untreated control plants (Fig. 2A). The PJA + HIN-treated and JA + HIN-treated plants showed significantly greater SOD activity in ICGV 86031, ICG 2271 and JL 24 ( $F_{5,17} = 11.7$ ,  $21.4$  and  $13.7$ , respectively,  $P < 0.01$ ) compared with the respective PSA + HIN-treated, SA + HIN-treated, HIN-treated and untreated control plants; however, in JL 24, SOD activity of PSA + HIN-treated and JA + HIN-treated plants did not differ significantly. Insect-resistant genotypes exhibited significantly greater SOD activity in all the treatments ( $F_{4,14} = 38.5$ ,  $21.4$ ,  $17.4$ ,  $25.6$  and  $13.6$  for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN and HIN, respectively,  $P < 0.05$ ) compared with JL 24. Untreated control plants did not show any significant difference across the genotypes.

### 3.1.6 APX activity

The APX activity of plants treated with PJA + HIN, PSA + HIN and JA + HIN was significantly greater ( $F_{5,17} = 38.5$ ,  $21.7$ ,  $37.3$ ,  $18.6$  and  $24.9$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.05$ ) than that of SA + HIN-treated, HIN-treated and untreated control plants (Fig. 2B). In ICG 2271, no significant difference was observed in APX activity of PSA + HIN-treated and SA + HIN-treated plants. Insect-resistant genotypes showed significantly greater APX activity in all the treatments ( $F_{4,14} = 30.3$ ,



**Figure 2.** Enzyme activities of groundnut plants pre- and/or simultaneously treated with JA and SA and infested with *H. armigera*: (A) superoxide dismutase (SOD) activity ( $\text{IU g}^{-1} \text{FW}$ ); (B) ascorbate peroxidase (APX) activity ( $\text{IU mg}^{-1} \text{protein}$ ); (C) catalase (CAT) activity ( $\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$ ); (D) *in vitro* protease inhibitor (PI) activity (%). Bars (mean  $\pm$  SD) of the same colour with similar letters within a genotype are not statistically different at  $P \leq 0.05$ . Asterisks on bars of the same colour show the significance across the genotypes within a treatment: \*\*, \*, = significant at  $P \leq 0.001$ , 0.01 and 0.05, respectively. PJA + HIN = pretreatment with JA 1 day prior to *H. armigera* infestation; PSA + HIN = pretreatment with SA 1 day prior to *H. armigera* infestation; JA + HIN = simultaneous application of JA and *H. armigera* infestation; SA + HIN = simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera*-infested plants.

21.1, 11.5, 9.3, 25.8 and 7.6 for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and untreated control, respectively,  $P < 0.05$ ) compared with that of the susceptible check JL 24.

### 3.1.7 CAT activity

The CAT showed altered expression in various treatments and in different genotypes (Fig. 2C). Significantly greater CAT activity was observed in plants infested with *H. armigera* and pre- and/or simultaneously treated with JA in groundnut genotypes ( $F_{5,17} = 33.9$ , 39.9, 28.5, 31.9 and 17.3 for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.01$ ) than in plants infested with *H. armigera* and pre- and/or simultaneously treated with SA and the untreated control plants, except in ICGV 86031, where CAT activity of PSA + HIN-treated plants was at par with that of JA + HIN-treated plants, and in JL 24, where no significant difference was observed in CAT activities of PSA + HIN-treated, JA + HIN-treated and SA + HIN-treated plants. The PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and untreated control plants of the insect-resistant genotypes showed significantly greater CAT activity ( $F_{4,14} = 11.3$ , 15.2, 8.6, 20.6, 17.2 and 10.5, respectively,  $P < 0.05$ ) than in JL 24.

### 3.1.8 PI activity

Significantly greater *in vitro* PI activity (%) was shown by groundnut plants treated with PJA + HIN and JA + HIN in ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24 ( $F_{5,17} = 47.1$ , 37.9, 32.2, 22.4 and 34.5, respectively,  $P < 0.05$ ) compared with PSA + HIN, SA + HIN, HIN and the untreated control plants (Fig. 2D). Across

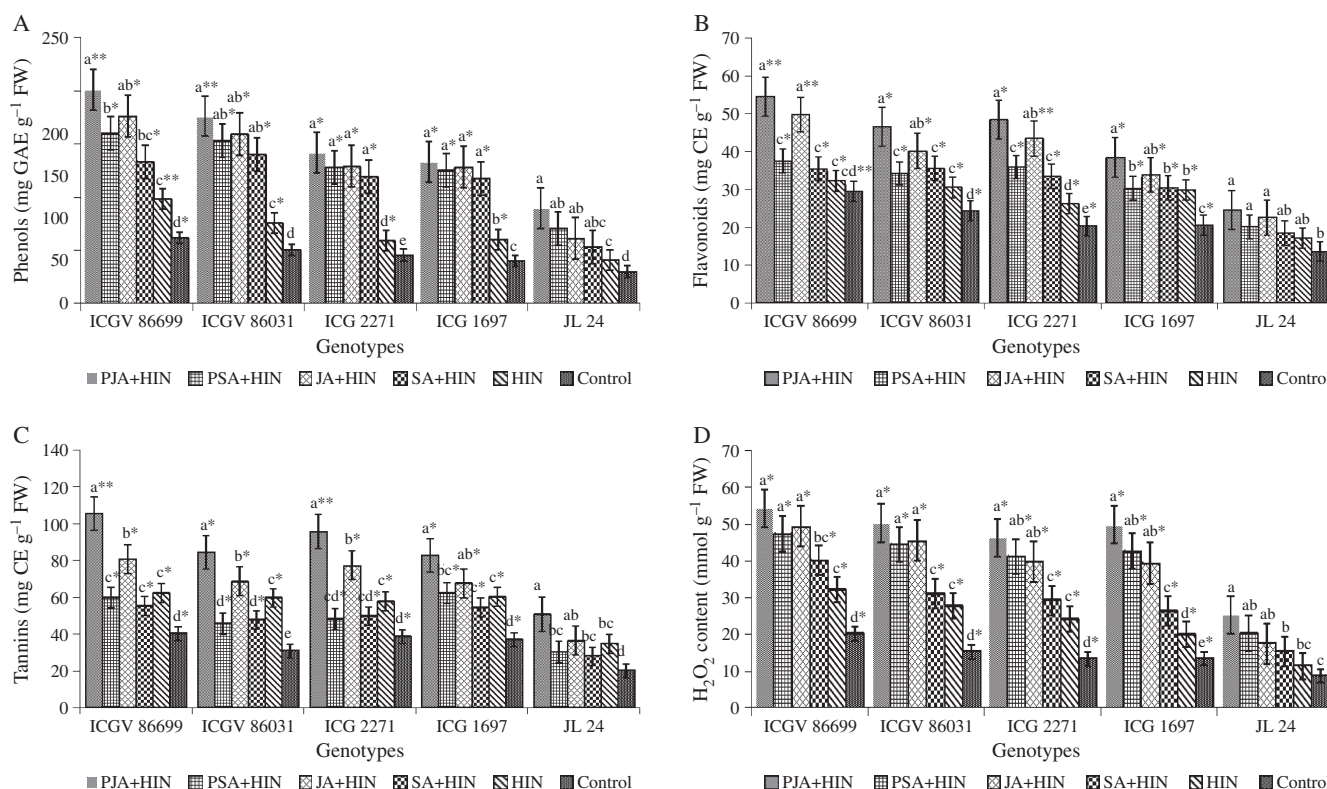
the genotypes, insect-resistant genotypes showed significantly greater PI activity in PJA + HIN-treated, PSA + HIN-treated, JA + HIN-treated, SA + HIN-treated and HIN-treated plants ( $F_{4,14} = 9.5$ , 11.7, 6.8, 8.1 and 10.2, respectively,  $P < 0.05$ ) than JL 24. No significant difference was observed in constitutive levels of PI activity across the tested genotypes.

### 3.1.9 Total phenols

There were no significant differences in phenolic content of the plants infested with *H. armigera* and pre- and/or simultaneously treated with JA and SA in ICGV 86031, ICG 2271, ICG 1697 and JL 24 ( $F_{5,17} = 30.4$ , 45.9, 28.3 and 39.8, respectively,  $P < 0.01$ ) (Fig. 3A). The PJA + HIN-treated and JA + HIN-treated plants of ICGV 86699 had significantly greater phenolic content ( $F_{5,17} = 30.4$ ,  $P < 0.05$ ) compared with the plants treated with PSA + HIN, SA + HIN and HIN and the untreated control plants; however, the phenolic content of plants treated with JA + HIN was at par with that of PSA + HIN-treated and SA + HIN-treated plants. The phenolic content of the insect-resistant genotypes was significantly greater in PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and untreated control plants ( $F_{4,14} = 25.4$ , 36.5, 29.7, 42.5, 30.6 and 31.2, respectively,  $P < 0.01$ ) compared with that of JL 24. The HIN-infested plants of ICGV 86699, ICGV 86031 and ICG 1697 had significantly higher phenolic content ( $F_{4,14} = 33.6$ ,  $P < 0.05$ ) than in ICG 2271 and JL 24.

### 3.1.10 Flavonoids

Flavonoid content was significantly greater in plants treated with PJA + HIN and JA + HIN in ICGV 86699, ICGV 86031 and ICG 2271



**Figure 3.** Amounts of plant secondary metabolites and other components of groundnut plants pre- and/or simultaneously treated with JA and SA and infested with *H. armigera*: (A) total phenols ( $\mu\text{g GAE g}^{-1}\text{ FW}$ ); (B) flavonoid content ( $\mu\text{g CE g}^{-1}\text{ FW}$ ); (C) condensed tannins ( $\mu\text{g CE g}^{-1}\text{ FW}$ ); (D)  $\text{H}_2\text{O}_2$  content ( $\mu\text{mol g}^{-1}\text{ FW}$ ). Bars (mean  $\pm$  SD) of the same colour with similar letters within a genotype are not statistically different at  $P \leq 0.05$ . Asterisks on bars of the same colour show the significance across the genotypes within a treatment: \*\*\*, \*\*, \* = significant at  $P \leq 0.001$ , 0.01 and 0.05, respectively. PJA + HIN = pretreatment with JA 1 day prior to *H. armigera* infestation; PSA + HIN = pretreatment with SA 1 day prior to *H. armigera* infestation; JA + HIN = simultaneous application of JA and *H. armigera* infestation; SA + HIN = simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera*-infested plants; GAE = gallic acid equivalents; CE = catechin equivalents.

( $F_{5,17} = 12.3, 17.5$  and  $10.9$  respectively,  $P < 0.01$ ) than in PSA + HIN-treated, SA + HIN-treated, HIN-treated and untreated control plants (Fig. 3B). In ICG 1697, the flavonoid content of JA + HIN plants was at par with that of PSA + HIN, SA + HIN and HIN plants. In JL 24, no significant differences were observed in flavonoid content of plants treated with PJA + HIN, PSA + HIN, JA + HIN, SA + HIN and HIN (Fig. 3B). Insect-resistant plants had greater amounts of flavonoids in all the treatments ( $F_{4,14} = 22.2, 13.5, 26.4, 14.9, 19.2$  and  $15.3$ , respectively, for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and untreated control,  $P < 0.05$ ) than JL 24.

### 3.1.11 Condensed tannins

There were significant differences in the condensed tannin content across the treatments and the genotypes tested (Fig. 3C). The PJA + HIN-treated plants exhibited greater levels of condensed tannins in ICGV 86699 ( $F_{5,17} = 35.7$ ,  $P < 0.01$ ), ICGV 86031 ( $F_{5,17} = 59.2$ ,  $P < 0.001$ ) and ICG 2271 ( $F_{5,17} = 27.9$ ,  $P < 0.05$ ) compared with PSA + HIN-treated, JA + HIN-treated, SA + HIN-treated, HIN-treated and untreated control plants. In ICG 1697 and JL 24, PJA + HIN-treated and JA + HIN-treated plants had significantly greater tannin content ( $F_{5,17} = 21.3$  and  $19.8$ , respectively,  $P < 0.05$ ) than PSA + HIN-treated, SA + HIN-treated, HIN-treated and untreated control plants. The tannin content of PSA + HIN plants was at par with that of JA + HIN in ICG 1697 and JL 24. Insect-resistant genotypes had significantly greater amounts of condensed tannins in all the treatments ( $F_{4,14} = 21.8, 11.7, 10.8, 16.5, 32.5$  and  $13.3$ , respectively, for PJA + HIN, PSA + HIN, JA +

HIN, SA + HIN, HIN and the untreated control,  $P < 0.05$ ) than the respective treatments in JL 24.

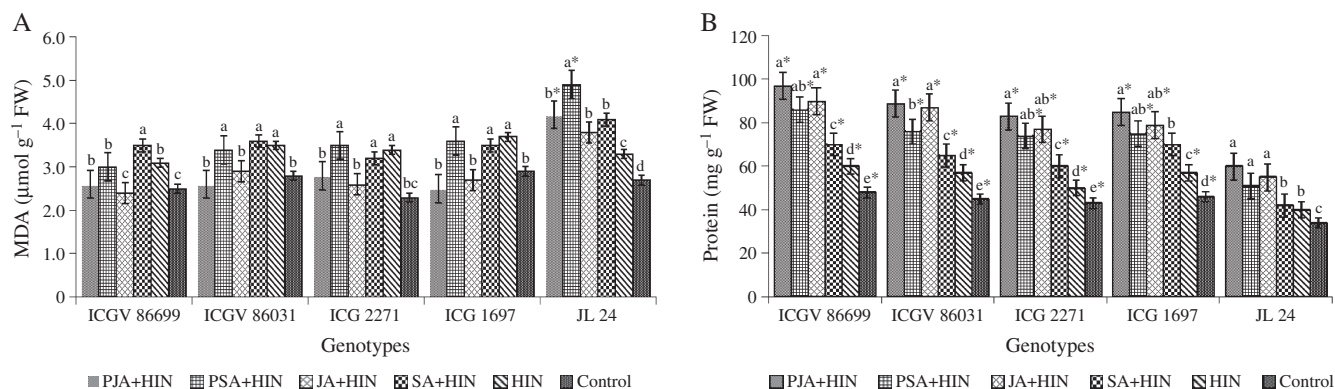
### 3.1.12 $\text{H}_2\text{O}_2$ content

The  $\text{H}_2\text{O}_2$  levels increased in plants in response to various treatments (Fig. 3D). The PJA + HIN-treated, PSA + HIN-treated and JA + HIN-treated plants had significantly greater  $\text{H}_2\text{O}_2$  content in ICGV 86699 ( $F_{5,17} = 27.9$ ,  $P < 0.001$ ), ICGV 86031 ( $F_{5,17} = 15.6$ ,  $P < 0.01$ ), ICG 2271 ( $F_{5,17} = 18.3$ ,  $P < 0.05$ ) and ICG 1697 ( $F_{5,17} = 9.3$ ,  $P < 0.05$ ) than the respective SA + HIN-treated, HIN-treated and untreated control plants. However, in JL 24, no significant difference was observed in  $\text{H}_2\text{O}_2$  contents of PSA + HIN-treated, JA + HIN-treated, SA + HIN-treated and HIN-treated plants. The insect-resistant genotypes showed considerable increase in  $\text{H}_2\text{O}_2$  content in all the treatments ( $F_{4,14} = 10.4, 15.7, 21.4, 13.9, 11.6$  and  $23.1$ , respectively, for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control,  $P < 0.01$ ) compared with JL 24.

### 3.1.13 MDA content

The MDA content varied between plants treated with JA and SA and insect-infested plants (Fig. 4A). The PSA + HIN-treated, SA + HIN-treated and HIN-treated plants exhibited greater MDA content in ICGV 86031, ICG 2271 and ICG 1697 ( $F_{5,17} = 10.3, 7.5$  and  $11.6$ , respectively,  $P < 0.05$ ) compared with PJA + HIN, JA + HIN and the untreated control plants. In ICGV 86699, the MDA content of plants treated with SA + HIN was significantly greater





**Figure 4.** Malondialdehyde (MDA) content ( $\mu\text{mol g}^{-1}$  FW) (A) and protein content ( $\text{mg g}^{-1}$  FW) (B) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application. Bars (mean  $\pm$  SD) of the same colour with similar letters within a genotype are not statistically different at  $P \leq 0.05$ . Asterisks on bars of the same colour show the significance across the genotypes within a treatment: \*\*\*, \*\*, \* = significant at  $P \leq 0.001$ , 0.01 and 0.05, respectively. PJA + HIN = pretreatment with JA 1 day prior to *H. armigera* infestation; PSA + HIN = pretreatment with SA 1 day prior to *H. armigera* infestation; JA + HIN = simultaneous application of JA and *H. armigera* infestation; SA + HIN = simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera*-infested plants.

( $F_{5,17} = 9.7$ ,  $P < 0.05$ ) than in the rest of the treatments. In JL 24, PSA + HIN-treated plants had a significantly greater MDA content ( $F_{5,17} = 18.3$ ,  $P < 0.05$ ) than PJA + HIN, JA + HIN, SA + HIN, HIN and the untreated control plants. PSA + HIN-treated, PJA + HIN-treated and JA + HIN-treated plants of JL 24 exhibited a significantly higher MDA content ( $F_{4,14} = 8.6$ , 11.1 and 7.8, respectively,  $P < 0.05$ ) than those of ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697. No significant differences were observed in the MDA content of PSA + HIN, SA + HIN, HIN and the untreated control plants across the genotypes.

### 3.1.14 Protein content

There was a tremendous increase in total protein content in JA- and SA-treated and insect-infested plants (Fig. 4B). The plants pretreated with JA and SA and infested with *H. armigera* and the plants treated with JA + HIN had a greater protein content ( $F_{5,17} = 12.6$ , 25.5, 21.3 and 6.6 for ICGV 86699, ICGV 86031, ICG 2271 and JL 24, respectively,  $P < 0.01$ ) than the plants treated with SA + HIN and HIN and the untreated control plants. There were no significant differences in protein content in ICG 1697 between JA + HIN-treated and SA + HIN-treated plants ( $P > 0.05$ ). Across the genotypes tested, the insect-resistant genotypes showed significantly greater accumulation of proteins ( $F_{4,14} = 21.4$ , 41.9, 33.4, 26.3, 16.9 and 9.5, respectively, for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control,  $P < 0.01$ ) than in the susceptible check JL 24.

### 3.2 Effect of JA- and SA-induced resistance on plant damage, larval survival and larval weights

The plant damage by *H. armigera* was significantly lower in plants pre- and/or simultaneously treated with JA in ICGV 86699 ( $F_{4,14} = 7.7$ ,  $P = 0.05$ ), ICGV 86031 ( $F_{4,14} = 10.5$ ,  $P < 0.05$ ) and ICG 1697 ( $F_{4,14} = 6.9$ ,  $P < 0.05$ ) compared with PSA + HIN, SA + HIN and the insect-infested plants (Table 1). In ICG 2271, no significant difference was observed in plant damage in PJA + HIN-treated, PSA + HIN-treated, JA + HIN-treated and SA + HIN-treated plants; however, it was significantly greater ( $F_{4,14} = 7.4$ ,  $P < 0.05$ ) than in HIN plants. Among the genotypes tested, the insect-resistant genotypes (ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697) suffered much lower damage in all the treatments compared with that of the susceptible check JL 24. There were significant differences in larval weights and larval survival across treatments. Larval survival

was significantly lower in PJA + HIN-treated plants in all the genotypes [ICGV 86699 ( $F_{4,14} = 15.7$ ,  $P = 0.05$ ), ICGV 86031 ( $F_{4,14} = 7.4$ ,  $P < 0.01$ ), ICG 2271 ( $F_{4,14} = 6.6$ ,  $P < 0.05$ ), ICG 1697 ( $F_{4,14} = 9.5$ ,  $P < 0.01$ ) and JL 24 ( $F_{4,14} = 5.5$ ,  $P < 0.01$ )]. Among the genotypes tested, the larvae fed on ICGV 86699 and ICGV 86031 showed significantly lower survivals ( $F_{4,14} = 11.9$ , 17.4, 9.3, 12.4 and 7.8, respectively, for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN and HIN, respectively,  $P < 0.05$ ) than those fed on JL 24 in all the treatments. Larvae fed on PJA + HIN-treated plants showed significantly lower weights ( $F_{4,14} = 23.3$ , 20.2, 15.3, 9.8 and 10.6 for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.01$ ) compared with those fed on PSA + HIN-treated, SA + HIN-treated, JA + HIN-treated and HIN-treated plants (Table 2). Across genotypes, larvae fed on ICGV 86699 had lower weights ( $F_{4,14} = 21.2$ , 11.4, 8.6 and 18.9 for PJA + HIN, PSA + HIN, JA + HIN and HIN, respectively,  $P < 0.05$ ) than those fed on the rest of the genotypes. However, no significant differences were observed between weights of the larvae fed on SA + HIN-treated plants of ICGV 86699 and ICGV 86031 ( $P > 0.05$ ).

## 4 DISCUSSION

Although several phytohormones are involved in host plant defence against biotic and abiotic stresses, JA and SA play an important role in modulating plant defence against insect herbivory.<sup>1,3–5,12</sup> The JA- and SA-mediated induced resistance operates through octadecanoid and phenylpropanoid pathways, respectively, resulting in increased production of secondary metabolites and plant volatiles.<sup>4,37</sup> JA also regulates the activity of calcium-dependent protein kinases involved in plant defence against a variety of biotic and abiotic stresses through signal transduction.<sup>38</sup> JA accumulates in plants in response to insect damage and also by exogenous application. During this process, several secondary metabolites and volatiles are produced.<sup>4</sup> Further, JA also activates antioxidative enzymes, such as POD, PPO and LOX, and the production of PIs.<sup>4</sup> SA regulates reactive oxygen species (ROS) metabolism in plants and the oxidation of certain substrates of POD, CAT, SOD and other antioxidative enzymes, thus altering the hormonal balance and cell-wall lignifications.<sup>3,10–12</sup> Increase in host plant resistance to herbivores has been observed through exogenous application of JA or MeJA<sup>4,37</sup> and SA.<sup>10,12</sup>

**Table 1.** Plant damage and *Helicoverpa armigera* larval survival on plants treated with jasmonic acid and salicylic acid<sup>a,b</sup>

Genotypes	Plant damage rating (DR) <sup>c</sup>					Survival (%)				
	PJA + HIN <sup>d</sup>	PSA + HIN <sup>e</sup>	JA + HIN <sup>f</sup>	SA + HIN <sup>g</sup>	HIN <sup>h</sup>	PJA + HIN	PSA + HIN	JA + HIN	SA + HIN	HIN
ICGV 86699	2.0 ± 0.9 c*	2.6 ± 0.5 b	2.4 ± 0.9 bc*	2.7 ± 0.4 b	3.2 ± 0.7 b	20.4 ± 2.1 c*	32.3 ± 2.3 bc	30.2 ± 4.6 c	36.5 ± 3.4 bc	41.2 ± 3.1 c
ICGV 86031	2.5 ± 0.8 bc*	3.0 ± 0.3 b	2.6 ± 0.8 b*	3.2 ± 0.6 b	3.5 ± 0.3 b	26.6 ± 2.1 bc*	34.3 ± 2.2 bc	35.5 ± 3.3 c	39.6 ± 4.4 bc	47.4 ± 2.1 b
ICG 2271	3.2 ± 0.9 b*	3.5 ± 0.3 b*	3.1 ± 0.6 b*	3.5 ± 0.7 b*	4.0 ± 0.6 b	32.4 ± 1.4 b*	40.5 ± 3.8 b	40.4 ± 2.1 b	44.5 ± 2.1 b	48.9 ± 3.1 b
ICG 1697	3.0 ± 0.7 b*	3.4 ± 0.6 b	3.0 ± 0.4 b*	3.6 ± 0.9 b	3.9 ± 0.7 b	35.7 ± 3.2 b*	44.8 ± 2.6 b	48.2 ± 3.2 b	50.5 ± 3.6 b	54.4 ± 4.7 b
JL 24	5.5 ± 1.1 a*	6.4 ± 1.1 a	6.2 ± 1.2 a	7.0 ± 0.6 a	7.5 ± 1.3 a	58.3 ± 2.1 a*	69.4 ± 3.8 a	75.9 ± 2.3 a	79.6 ± 4.1 a	81.4 ± 6.6 a

<sup>a</sup> Values (mean ± SD) followed by the same letter(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).<sup>b</sup> An asterisk (\*) in a row shows significant difference in plant damage and larval survival across the treatments within a genotype.<sup>c</sup> DR = *Helicoverpa* damage rating to a scale of 1–9 (1 ≤ 10% and 9 ≥ 80%) 6 days after infestation.<sup>d</sup> PJA + HIN = pretreatment with JA 1 day prior to *H. armigera* infestation.<sup>e</sup> PSA + HIN = pretreatment with SA 1 day prior to *H. armigera* infestation.<sup>f</sup> JA + HIN = simultaneous application of JA and *H. armigera* infestation.<sup>g</sup> SA + HIN = simultaneous application of SA and *H. armigera* infestation.<sup>h</sup> HIN = *H. armigera*-infested plants.**Table 2.** Weight (mg) of *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants<sup>a,b</sup>

Genotypes	Treatments				
	PJA + HIN <sup>c</sup>	PSA + HIN <sup>d</sup>	JA + HIN <sup>e</sup>	SA + HIN <sup>f</sup>	HIN <sup>g</sup>
ICGV 86699	37.5 ± 3.1 d*	48.6 ± 5.3 d	47.5 ± 5.6 d	59.7 ± 3.5 e	69.6 ± 3.6 d
ICGV 86031	44.5 ± 2.8 bc*	60.6 ± 3.7 c	75.5 ± 7.7 bc	74.4 ± 3.7 de	97.7 ± 5.3 c
ICG 2271	55.4 ± 3.2 b*	65.6 ± 5.3 c	87.6 ± 3.4 b	98.8 ± 4.7 bc	110.3 ± 8.8 bc
ICG 1697	59.6 ± 2.7 b*	80.6 ± 6.4 b	95.5 ± 4.3 b	114.4 ± 6.3 ab	127.5 ± 7.3 b
JL 24	73.6 ± 4.3 a*	102.4 ± 7.6 a	120.3 ± 8.7 a	129.5 ± 9.5 a	159.5 ± 10.0 a

<sup>a</sup> Values (mean ± SD) followed by the same letter(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).<sup>b</sup> An asterisk (\*) in a row shows significant difference in larval weight across the treatments within a genotype.<sup>c</sup> PJA + HIN = pretreatment with JA 1 day prior to *H. armigera* infestation.<sup>d</sup> PSA + HIN = pretreatment with SA 1 day prior to *H. armigera* infestation.<sup>e</sup> JA + HIN: simultaneous application of JA and *H. armigera* infestation.<sup>f</sup> SA + HIN = simultaneous application of SA and *H. armigera* infestation.<sup>g</sup> HIN = *H. armigera*-infested plants.

Elucidation of various defensive responses in plants by exogenous application of JA and SA is essential for gaining an understanding of the induced plant resistance against insect pests that is mediated by these hormones and the implications for insect pest management.

The present results showed that plants pretreated with JA had greater activity of defensive enzymes such as POD and PPO than the plants pretreated with SA. Increase in POD activity is regarded as the initial response of plants to insect attack.<sup>5,8</sup> Increased activities of these enzymes in response to JA might be due to the greater accumulation of JA after insect infestation, and the subsequent activation of plant defensive pathways, resulting in increased activity of defensive enzymes such as POD and PPO. Higher levels of POD activity enhance cell lignification, wound healing and production of secondary metabolites, besides detoxifying the peroxides, thus defending the plants against insects, pathogens and other stresses.<sup>8,39,40</sup> The reduced nutritional quality of plant tissues on account of PPO has also been reported to play an important role in plant defence against insect herbivory.<sup>10,41,42</sup> Moreover, toxic but highly reactive quinines produced from phenol oxidation interact with the nucleophilic side chain of amino acids and crosslink the proteins in plant tissues, thus reducing their digestibility.<sup>42</sup>

PAL activity is induced by various stresses, including insect herbivory.<sup>10</sup> PAL activity was greater in groundnut plants pretreated with JA and SA and in plants simultaneously treated with JA compared with insect-infested and uninfested control plants. The increase in PAL activity by JA and SA can be attributed to their identical effect on the activation of defensive pathways in response to damage by *H. armigera*. These pathways produce various plant secondary metabolites, which on oxidation form several defensive compounds.<sup>10</sup> In addition, the phenylpropanoid pathway, of which PAL is a central enzyme, also leads to lignin synthesis.<sup>43</sup> Lipoxygenase gene expression is regulated by JA and different biotic/abiotic stresses, including insect herbivory.<sup>44</sup> LOX catalyses the production of JA from linolenic acid in the octadecanoid pathway.<sup>45</sup> It also elicits the production of various plant defensive secondary metabolites and plant volatiles. The present study revealed that PJA + HIN-treated and JA + HIN-treated plants had significantly greater levels of LOX activity than the rest of the treatments. This increased LOX activity in plants pre- and/or simultaneously treated with JA might be due to signalling of the octadecanoid pathway by exogenous application of JA. Oxylinins produced from fatty-acid oxidation by LOX play a wide array of functions in plant growth and development, senescence and defence against biotic and abiotic stresses, including insect herbivory.<sup>46</sup> Compounds formed from LOX-mediated reactions



are either directly deterrent to insect pests and/or produce post-ingestive toxicity in Insects.<sup>45</sup>

The antioxidative enzymes involved in plant oxidative stress due to biotic and abiotic factors are SOD, APX and CAT. The present study revealed greater increase in APX activity in plants pretreated with JA and SA, and JA + HIN. Insect-resistant genotypes exhibited significantly greater APX activity than the susceptible check JL 24. Pretreatment with JA followed by insect infestation and simultaneous application of JA and insect infestation resulted in greater increase in CAT and SOD activities across the genotypes. Pre- and/or simultaneous treatment with SA also increased the activities of these enzymes; however, induction was lower compared with that of JA. Insect-resistant genotypes showed greater increase in the activities of antioxidative enzymes compared with the susceptible check JL 24, but the levels of induction varied. The differential responses across the genotypes might be due to the differential ability of groundnut genotypes to perceive insect damage and/or the ability to mount a defensive response. Greater increase in SOD, APX and CAT following JA or SA treatment could be due to signalling of transduction pathways modulated by these phytohormones, which leads to the production of antioxidative enzymes to scavenge the toxic-free radicals produced by herbivory. The higher constitutive levels of these enzymes in insect-resistant genotypes might protect them from initial oxidative damage before the induced defence system is activated. APX decreases the ascorbate content in plant tissues by utilising ascorbic acid as the electron donor in ascorbate-glutathione recycling while catalysing the reduction of H<sub>2</sub>O<sub>2</sub> to water, which in turn reduces insect growth and development.<sup>47</sup> Greater APX activity in soybean leaves removes ascorbate from the *H. zea* larval midgut, thereby reducing insect growth and development.<sup>47</sup> *Scirpophaga incertulas* (Walk.) and *Cnaphalocrosis medinalis* (Guenee) damage induces higher levels of CAT in rice.<sup>48</sup> CAT resists the oxidative stress in soybean caused by *H. zea* infestation.<sup>49</sup> SOD converts the toxic-free radicals, especially of oxygen, into less toxic and relatively stable H<sub>2</sub>O<sub>2</sub>.<sup>50</sup> Induction of SOD activity by SA has been found to reduce plant oxidative damage in maize.<sup>51</sup> *H. zea* infestation increases SOD activity in tomato<sup>52</sup> and soybean.<sup>49</sup>

Plants produce many non-enzymatic defensive proteins against insect pests. However, PIs are the most exploited plant defensive proteins that confer resistance to insect pests.<sup>53</sup> The *in vitro* PI activity of groundnut plants pre- and/or simultaneously treated with JA and infested with *H. armigera* was significantly greater than that of uninfested control plants. Overall, insect-resistant genotypes showed greater PI activity than JL 24 in almost all the treatments. The reduction in protein digestibility by PIs and deprivation of insects of essential amino acids lead to retarded growth and development of Insects.<sup>53</sup> PIs are strongly upregulated in plants in response to wounding or herbivore damage and/or elicitor application. For example, exogenous application of MeJA in *Nicotina attenuata* Torr. ex S. Watson results in quick accumulation of JA and the induction of trypsin proteinase inhibitors against *M. sexta*.<sup>54</sup>

Phenols constitute one of the most important and extensively studied groups of secondary metabolites against insect pests.<sup>7,17,48</sup> An abrupt increase in phenolic content occurs in plants damaged by insects and/or treated with elicitors, including JA and SA.<sup>21,22</sup> PJA + HIN-treated, PSA + HIN-treated and HIN-treated plants exhibited greater phenolic content than the SA + HIN-treated and untreated plants; however, some genotypes, such as ICG 2271, ICG 1697 and JL 24, responded similarly to pre- and/or simultaneous treatments of JA and SA. Further,

insect-resistant genotypes showed a greater increase compared with the susceptible check JL 24. This might be due to the strong induction of the octadecanoid and phenylpropanoid signalling pathways by JA and SA, respectively. Flavonoids have been reported to confer resistance against *Spodoptera frugiperda* (J.E. Smith) in *Arabidopsis thaliana* (L.).<sup>55</sup> Higher levels of flavonoids, such as daidzein and genistin, have been observed in soybean plants infested with *Nezara viridula* (L.).<sup>56</sup> Tannins have been reported to be systemically induced in insect-damaged plants.<sup>54</sup> In *N. attenuata*, application of MeJA induced greater accumulation of JA, which in turn activated the production of phenols, flavonoids, nicotine and trypsin proteinase inhibitors against *M. sexta*.<sup>54</sup>

The oxidative state of the host plants is associated with plant resistance to insects,<sup>5,10</sup> which results in the production of ROS, which are toxic to herbivores. The present results showed that both JA and SA induced higher levels of H<sub>2</sub>O<sub>2</sub> in all the genotypes infested with *H. armigera*. However, the induction was greater in plants pretreated with JA and SA and in plants simultaneously treated with JA and infested with *H. armigera*. Insect-resistant genotypes showed a strong response in terms of accumulation of H<sub>2</sub>O<sub>2</sub>. The higher induction of H<sub>2</sub>O<sub>2</sub> by pretreatment with JA and SA could be attributed to the increased activity of antioxidative enzymes in the treated plants, and conversion of toxic-free radicals into H<sub>2</sub>O<sub>2</sub>. JA and SA induce oxidative burst in plants,<sup>10–12</sup> which happens to be the first and foremost defence against insect herbivory.<sup>5,8,17,48</sup> Transduction pathways signalled by H<sub>2</sub>O<sub>2</sub> produce many defensive compounds, which results in the oxidation of phenols and other compounds producing many defensive compounds.<sup>11</sup> Oxidative damage in the midgut of insects feeding on pre-wounded plants is due to the accumulation of H<sub>2</sub>O<sub>2</sub> through JA- and SA-mediated pathways.<sup>12,57</sup>

Malondialdehyde is an important lipid peroxidation product that indicates the extent of plant defensive response to stress. The plants infested with *H. armigera* and pre- and/or simultaneously treated with SA had a higher MDA content. Overall, JL 24 showed higher amounts of MDA among all the genotypes. This could be due to greater stress experienced by this genotype and the higher levels of lipid peroxidation. Lipid peroxidation and hydroxyl ion formation (OH<sup>•</sup>) have been proposed to play an important role in plant defence by increasing the activity of oxidative enzymes.<sup>49</sup> MDA is also involved in volatile emission, and thus has a role in indirect plant defence as well.<sup>58</sup> Hao *et al.*<sup>59</sup> reported higher amounts of MDA in rice plants in response to rice stripe virus and small brown planthopper, *Nilaparvata lugens* (Stål.). Induction of proteins and their role in induced resistance against insect pests have been well established.<sup>5,41,48</sup> The present studies indicated that there was a significant increase in proteins in plants treated with PJA + HIN, followed by JA + HIN-treated plants. Increase in protein concentration may be due to the increase in antioxidative enzymes and other non-enzymatic defensive proteins. Defence-related enzymes and other protein-based defensive compounds accumulate in plants in response to oxidative stress<sup>39,41</sup> and on the application of elicitors,<sup>4,21,22,37</sup> which defend them from various biotic and abiotic stresses.

Expression of resistance to insects and insect growth and development are closely related. The PJA + HIN-treated plants suffered lower damage due to *H. armigera* across genotypes. The insect-resistant genotypes showed greater reduction in plant damage than the susceptible check JL 24. Similar results were observed in terms of larval survival and larval weights of *H. armigera*. Reduced damage and lower larval survival and larval

weights might be due to the greater production of toxic secondary metabolites in the insect-resistant genotypes by insect damage and JA application.<sup>41,42,44,45</sup> Reduced damage and lower larval growth and development were correlated with increased activity of POD, PPO and other defensive enzymes induced following insect attack and/or elicitor application. Larvae of *Manduca sexta* (L.) and *Spodoptera exigua* (Hüb.) fed on JA-deficient mutant (*def1*) tomato plants exhibited higher survival and weight gain compared with those fed on wild-type tomato.<sup>60,61</sup> Increased levels of POD, PPO and LOX in plants have been correlated with reduction in insect growth and development.<sup>39,42,52</sup> Plant defensive compounds induced in insect-resistant genotypes reduced the survival and development of *S. frugiperda* larvae.<sup>41</sup> Reduced larval weights due to antibiosis and antixenosis against *H. armigera* have also been observed in chickpea.<sup>13</sup>

## 5 CONCLUSIONS

The present study has shown that both JA and SA induce antioxidative responses in groundnut plants against *H. armigera*, which in turn reduce insect growth and development; however, the effect of JA is greater than the effect of SA. The insect-resistant genotypes have a better capability to respond to exogenous application of JA and SA than the susceptible check JL 24. JA results in greater induced response than SA. The results suggest that induced resistance can be exploited as a component of pest management.

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